ABSTRACT A new concept of a basic mechanism involved in cell proliferation is presented. It is suggested that cells are normally restrained from proliferating by the highly viscous nature of the intercellular glycosaminoglycans. In order to proliferate, cells must escape from this restraint by depolymerizing the glycosaminoglycans in their immediate environment. This process is accomplished by the release of hyaluronidase and kept in check by a physiological hyaluronidase inhibitor. It is suggested that physiological hyaluronidase inhibitor is an oligoglycosaminoglycan that requires ascorbic acid for its synthesis, and perhaps incorporates residues of ascorbic acid. This hypothesis provides a comprehensive explanation for many disease processes involving cell proliferation. It indicates the existence of a basic underlying mechanism and suggests a common pattern of treatment for these diseases, including cancer. We conclude that ascorbic acid may have much greater therapeutic value than has been generally assigned to it.

INTRODUCTION

Orthomolecular medicine is the preservation of good health and the treatment of disease by varying the concentrations in the human body of substances that are normally present in the body and are required for health (1, 2, 3). Of these substances, the vitamins are especially important, and ascorbic acid, in particular, may have much greater value than has been previously ascribed to it. Irwin Stone has advanced arguments to support the concept that the optimum rate of intake of ascorbic acid is about 3 g per day under ordinary conditions, and larger, up to 40 g per day, for a person under stress (4, 5, 6). An argument based on the fact that only a few ~~animals~~ animal species require an exogenous source of ascorbic acid and on the amounts of ascorbic acid contained in a diet of raw natural plant food has led to the conclusion that the optimum daily intake of ascorbic acid for an adult human being is about 2.3 g or more (7). Ascorbic acid is a substance with extremely low toxicity; many people have ingested ten to twenty grams per day for long periods without serious side effects, and ingestion of as much as ~~100~~ 150 grams ~~at one time~~ within 24 hours without serious side effects has been reported (8). In this respect ascorbic acid may be considered an ideal substance for orthomolecular prophylaxis and therapy.

GROUND SUBSTANCE

Cells in the tissues of the body are embedded in ground substance. This ubiquitous material pervades every interspace and isolates every cell from its neighbors. It must be traversed by every molecule entering or leaving the cell. There is evidence that the interface between a cell membrane and the immediate extracellular environment is the crucial factor in the whole proliferative process. Variations in the composition of the extracellular environment exert a profound influence on the cell behavior, and in turn the cells possess a powerful means of modifying their immediate environment. This interdependence is involved in all forms of cell proliferation and is particularly important in cancer.

The cancer cell and its immediate environment constitute a balanced system in which each component influences the other. Understanding of this relationship and of the means of controlling it could lead to rational methods of treating cancer and other cell-proliferative diseases.

THE INTERCELLULAR ENVIRONMENT

Until recently cancer research has tended to concentrate almost exclusively upon the cell, and to ignore the other half of the proliferation equation. The intercellular ground substance is a complex gel, containing water, electrolytes, metabolites, dissolved gases, trace elements, vitamins, enzymes, carbohydrates, fats, and proteins. The solution is rendered highly viscous by an abundance of certain long-chain acid mucopolysaccharide polymers, the glycosaminoglicans and the related proteoglycans, reinforced at the microscopic level by a three-dimensional network of collagen fibrils. The principal glycosaminoglycans so far identified are hyaluronic acid, a long-chain polymer with high molecular weight (200,000 to 500,000) and simple chemical structure (alternating residues of N-acetylglucosamine and glucuronic acid), and varieties of chondroitin (alternating residues of N-acetylgalactosamine and glucuronic acid) and its sulfate esters. Other glycosaminoglycans may also be present. The chemistry of the ground substance and the intercellular environment has been reviewed recently by Balazs (1, 9).

An important property of the intercellular substance is its very high viscosity and cohesiveness. This property is dependent upon the chemical integrity of the large molecules. The viscosity can be reduced and the structural integrity destroyed by the depolymerizing (hydrolyzing) action of certain related enzymes (the endohexosaminidases, the beta-N-acetylglucosaminidases, the beta-N-acetylgalactoseaminidases, and beta-glucuronidase), known by the generic name "hyaluronidase". It is probable that most cells in the body are able to produce hyaluronidase (10, 11). The interlacing molecular network of the intercellular ground substance is in a constant state of slow dynamic change, with synthesis of glucosaminoglycans balanced by

their breakdown (~~depolymerization~~) ~~by~~ through the catalytic by hydrolysis action of hyaluronidase, and subsequent excretion. It is within this slowly changing environment, the "milieu interieur" of Claude Bernard, that all cellular activity takes place. The normal cell and the cancer cell both thrive and die within this environment.

HYALURONIDASE AND CELL PROLIFERATION

Some years ago the hypothesis was advanced that all forms of cell proliferation depend upon one fundamental interaction between the cell and its immediate environment (12). The hypothesis may be stated as follows: All cells in the body are embedded in a highly viscous environment of ground substance which physically restrains their inherent tendency to proliferate; Proliferation is initiated by release of hyaluronidase from the cells, which catalyzes the hydrolysis of the glycosaminoglicans in the immediate environment and allows the cells freedom to divide and to migrate within the limits of the alteration; proliferation continues as long as hyaluronidase is being released, and stops when the production of hyaluronidase stops or when the hyaluronidase is inhibited, and the environment is allowed to revert to its normal restraining state.

NORMAL CELL PROLIFERATION

In normal healthy tissues cell division is taking place at a constant slow rate, corresponding to normal cell replacement. This normal "background" rate of cell division results in a slow metabolic turnover of ground substance, with liberation into the blood stream and then escape in the urine of the partially depolymerized fractions of the intercellular glycosaminoglicans, produced in the immediate vicinity of the dividing cells. These degradation products of ground substance depolymerization can be recognized and measured by a variety of biochemical methods. Depending upon the analytical procedure employed, different fractions have been given different names. For our purpose they may be grouped together under the general term "serum polysaccharide". In health the serum polysaccharide concentration remains within a relatively narrow "normal" range (13,14). The process is kept in check by the presence in the tissues and the blood of a substance called "physiological hyaluronidase inhibitor" (PHI). In health the serum PHI concentration lies within a well-defined "normal" range (15,16).

EXCESSIVE CELLULAR PROLIFERATION

In conditions in which excess cell proliferation is occurring, such as inflammation, tissue repair, and cancer, depolymerization of ground substance can be demonstrated histochemically in the immediate vicinity of the proliferating cells (17), and there is also a significant increase in concentration of both the serum polysaccharide (13,14) and the serum PHI (15,16).

NEOPLASTIC CELL PROLIFERATION

It follows from this hypothesis that cancer may be no more than the permanent exhibition by some cells of a fundamental biological property possessed by all cells. We suggest that the characteristic feature of neoplastic cell proliferation is that these cells in becoming malignant have acquired, and are able to bequeath to their descendants in perpetuo, the ability to produce hyaluronidase continuously. Wherever they travel, these cells will always prosper, multiply, and invade within the protective independence of their own self-created depolymerized environment. These renegade cells are autonomous only because they possess this specific ability, the ability to isolate themselves permanently from "contact" and all the usual "controls" governing tissue organization and growth restraint.

By endowing a clone of cells with this single property of continuous hyaluronidase release it is possible to provide a reasonable explanation for many of the morphological features of malignant invasive growth (12). The methods whereby cells might acquire this property in response to a wide variety of carcinogenic stimuli have also been outlined, together with the experimental evidence in support of the concept (12).

THERAPEUTIC CONTROL OF CELL PROLIFERATION

Assuming that cellular proliferation depends upon the interaction between depolymerization of the ground substance and its inhibition by cellular hyaluronidase, we see that there are two methods of exerting therapeutic control of cancer and of other disease states in which excessive cellular proliferation is a harmful feature. We may attempt to increase the resistance of the ground substance to enzymatic depolymerization, that is, to strengthen the ground substance, or to directly neutralize the cellular hyaluronidase by decreasing its production or inhibiting its action.

TREATMENT BY STRENGTHENING THE GROUND SUBSTANCE

The resistance of ground substance to the action of hyaluronidase can be increased in a number of ways, some of which are already established as useful methods for retarding cell proliferation.

Radiotherapy, irradiation with x-rays, is an example in which the result of the treatment is that some of the amorphous ground substance has been replaced by a dense deposit of collagen (13). The direct cytotoxic effect of radiotherapy is less powerfully reinforced by a permanent reduction in the susceptibility of the ground substance in the treated region to the action of hyaluronidase, with a consequent long-lasting diminution in proliferative activity.

Hormone therapy is effective because the physical-chemical state of the ground substance is profoundly influenced by many endocrine factors; the experimental evidence has been reviewed elsewhere (12). Resistance to the action of hyaluronidase can be increased by the administration of corticosteroids, estrogens, androgens, and thyroxine, and these effects are enhanced after adrenalectomy and hypophysectomy. These hormones, although differing widely in their special effects on particular target cells, all exert to a greater or lesser extent, and roughly in the order stated, the same effect on the intercellular field, namely, the absorption of amorphous ground substance and its replacement by a more resistant fibrous substance. These hormones are the ones employed with some success in the palliation of various forms of human cancer.

Other agents may also be effective in altering the intercellular environment and indirectly exerting some controlling influence on the behavior of cells. It has been pointed out (12) that an explanation is provided of the "Haddow paradox", that substances which are locally carcinogenic (by creating a local intensely impermeable carcinogenic environment) have also some anti-proliferative value when administered systemically in experimental cancer (by bringing about similar generalized changes in the resistance of the ground substance to hyaluronidase and thus decreasing cell proliferation).

Because of the complexity of the intercellular ground substance and its responsiveness to external influences, many of the innumerable "cancer treatments" that have been hopefully advocated year after year might have some element of truth behind them. It is also true, however, that no form of cancer treatment based on the antineoplastic effect of modification of ground substance can ever be more than palliative, because to render the ground substance totally resistant to hyaluronidase would create a situation incompatible with life itself.

TREATMENT BY INHIBITION OF HYALURONIDASE

Although the indirect methods of retarding cell proliferation, described above, are of great interest and in special circumstances of undoubted value, the direct inhibition of cellular hyaluronidase offers more spectacular therapeutic possibilities.

Hyaluronidase may be inhibited by drugs and by immunological methods, but the approach most likely to succeed appears to be that of utilization of the naturally occurring PHI substance.

Spontaneous regression of advanced cancer has been well documented in a number of fortunate patients as a direct consequence of massive intercurrent infection with hyaluronidase-producing bacteria (19). A possible explanation for this remarkable phenomenon is that the depolymerizing action evoked an upsurge in the serum PHI level of sufficient magnitude to inhibit totally the malignant capability of the neoplastic cells (12). It is known that such infections are always associated with an increase in the serum PHI concentration (15,16). It has been independently demonstrated in experimental cancer that the injection of Shear's polysaccharide (5) not only induces carcinolysis (20) but also a sharp and significant rise in PHI concentration (21). The problem is how to employ this suppressive mechanism in practical therapeutics.

As ascorbic acid and hyaluronidase inhibitor

ASCORBIC ACID AND HYALURONIDASE INHIBITOR

There is strong evidence to indicate that ascorbic acid is involved in some way in the synthesis of physiological hyaluronidase inhibitor. A strong suggestion to this effect is provided by the manifestations of scurvy, resulting from a deficiency of ascorbic acid. If ascorbic acid were required for the synthesis of PHI, a deficiency of ascorbic acid would cause the serum PHI level to decrease toward zero. In the absence of the control of hyaluronidase by PHI, background cellular proliferation and release of more hyaluronidase would produce a steady and progressive enzymatic depolymerization of the ground substance, with disruption and disintegration of the collagen fibrils, intraepithelial cements, basement membranes, perivascular sheaths, and all the other organized cohesive structures of the tissues, producing in time the generalized pathological state of scurvy. These generalized changes, of tissue disruption, ulceration, and hemorrhage, are identical to the local changes that occur in the immediate vicinity of invading neoplastic cells. This concept of scurvy, as involving uncontrolled depolymerization of ground substance, explains why scurvy is always associated with a very high level of serum polysaccharide (22). It also explains why very small amounts of ascorbic acid have profound effects in the treatment of scurvy. The total body content of ascorbic acid is estimated to be around 5 g (23), and yet this small amount controls the health of the whole body content of intercellular material, which must amount to many kilograms of substance. The symptoms of scurvy are relieved by the ingestion of a few tenths of a milligram of ascorbic acid. It seems clear that ascorbic acid is not an important constituent unit of the intercellular ground substance, as has been suggested; instead, it may well be involved in the synthesis of PHI, the circulating factor that controls intercellular homeostasis. Ascorbic acid is, of course, required for the conversion of proline to hydroxyproline, and is accordingly essential for the synthesis of collagen. It may well serve

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in several ways in determining the nature of tissues and the state of health of human beings.

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PHI ~~is a long-chain glycosaminoglycan polymer with smaller molecular weight but~~ has a general chemical similarity to the glycosaminoglycan polymers of the ground substance (~~14~~ 15, 16). The PHI serum concentration rises significantly in all conditions in which excessive cell proliferation is a feature (~~14~~ 15), but PHI is known not to be a simple breakdown product of ground-substance glycosaminoglycan. Its precise chemical composition is still unknown. It has recently been suggested (~~23~~ 25) that PHI is a soluble glycosaminoglycan residue in which some or all of the glucuronic acid units are replaced by the somewhat similar molecules of ascorbic acid. The general theory of enzyme activity and the action of inhibitors (~~24~~ 26) involves the idea that the active region of the enzyme is complementary to the intermediate complex, with the structure corresponding to the maximum of the energy curve (~~the~~ at the saddle-point configuration, intermediate ~~between~~ in structure between the reactants and the products) that determines the rate of reaction. This theory requires that inhibitors of the enzyme resemble the activated complex, rather than either the reactant molecules or the product molecules. Accordingly it is unlikely that PHI would be ~~closely similar in structure to~~ a fragment of hyaluronic acid or a fragment of any other glycosaminoglycan. It would instead involve at least one residue of a related but different substance. It is possible that a residue of ascorbic acid resembles the activated complex, and that incorporation of such a residue would produce an altered glycosaminoglycan ~~with~~ which could function as an inhibitor of hyaluronidase. It is also possible, however, that the chemical activity of ascorbic acid, such as its reducing power ~~which might introduce a double bond in the chain~~, or its power to cause hydroxylation reactions to take place, could function to convert an oligoglycosaminoglycan into PHI. Whatever the mode of action of ascorbic acid in synthesis of PHI, whether it involves incorporation of an ascorbic acid residue or some other reaction, the therapeutic implications of the concept that ascorbic acid is involved in the synthesis of PHI are considerable.

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A preparation of PHI has been found to ~~contain~~ have molecular weight about 100,000 and to consist of 94 percent protein and 6 percent polysaccharide (24). It is our opinion that it is the polysaccharide that has the power of combining with the active region of PHI and inhibiting its enzymatic activity, and the following discussion is based on that opinion.

(To be introduced earlier)

The hypothesis that ascorbic acid is required for the synthesis of PHI and is itself destroyed in the course of the synthesis would explain why in such conditions as inflammation, wound repair, and cancer the individual always appears to be deficient in ascorbic acid, on the basis of measurement of level in the serum, measurement of urinary excretion, and saturation tests (27, 28, 29, 30). It is clear that the total body requirement of ascorbic acid has become abnormally high, as would result from an increased synthesis of PHI with incorporation or destruction of ascorbic acid.

THE SYNTHESIS OF PHI AND ITS THERAPEUTIC USE

If the basic concept of cellular proliferation is correct, PHI might be a valuable therapeutic agent in directly controlling all forms of excessive proliferation, including cancer. It is a naturally occurring substance found in the serum of all mammals (24), and should be safe and free from dangerous side effects. Determination of the chemical structure of PHI and its synthesis should not present insuperable difficulties. However, it may not be necessary to synthesize the substance. It is possible that, given enough ascorbic acid, the body could synthesize a proper quantity of PHI.

The level of ascorbic acid in blood plasma is about 15 mg per liter when the rate of intake is about 200 mg per day. With larger rates of intake the plasma level increases only slowly, because of urinary excretion, reaching about 30 mg per liter for intake of 10 g per day. In the therapeutic situations here envisaged, with ascorbic acid being prescribed to control excessive cell proliferation, a daily intake of 10 g to 20 g or even more, and with the bulk of that administered intravenously at first, might be necessary to achieve the desired effect. Cameron and McCormick (28,29) and Klenner (14) have been advocating and using this form of treatment for many years. Their combined clinical experience indicates that very large doses of ascorbic acid, in the range mentioned above, can be given intravenously with perfect safety and with apparent benefit in a wide variety of disease states.

THE THERAPEUTIC USES OF ASCORBIC ACID

The hypothesis that ascorbic acid is required for synthesis of PHI and can thus control harmful depolymerization of glycosaminoglycans explains why the vitamin is effective in curing scurvy and in improving the healing of wounds. But the potential therapeutic uses of this relatively simple substance may be infinitely greater.

It has been postulated for years that the administration of ascorbic acid would increase tissue resistance to bacterial and viral infections by improving the integrity of the tissues. We are now in a position to suggest that, through the PHI system, the administration of ascorbic acid in sufficiently high dosage may provide us with a broad-spectrum antibiotic effective against all those pathogenic bacteria, and perhaps viruses, that rely upon release of hyaluronidase to establish and spread themselves throughout the tissues. The dramatic clinical successes reported quite independently by McCormick (16,17) and by Klenner (18) in a very wide variety of infective states support this contention.

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The hypothesis also indicates a safe and elegant method of control in many inflammatory and auto-immune diseases where, although the individual causes are still unknown, the essential harmful feature is always excessive cell proliferation and ground-substance depolymerization. A trial of orthomolecular doses of ascorbic acid seems perfectly justifiable and eminently preferable to the use of corticosteroids, irradiation, and all the other indirect methods currently employed.

Most important of all, we are led to the conclusion that the administration of this harmless substance, ascorbic acid, might provide us with an effective means of permanently suppressing neoplastic cellular proliferation and invasiveness, in other words, an effective means of controlling cancer. Ascorbic acid in adequate doses might prove to be the ideal cytostatic agent. Dramatic regressions might be induced in a

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The effectiveness of the water-soluble anti-oxidant ascorbic ground substance and especially cell membranes may be increased by the simultaneous administration of the fat-soluble anti-oxidant vitamin E.

very few patients with rapidly growing tumors with precarious blood supplies, but in the great majority the effect of the treatment is expected to be to "disarm" rather than to "kill" the malignant cells. "Tumors" would remain palpable and visible on x-ray examination, but all further progressive malignant growth might be stopped. Hopefully, malignant ulcers would heal, and pain, hemorrhage, cachexia, and all the other secondary distressing features of neoplasia would be brought under control. This desirable outcome might be termed carcinostasis, with what had been neoplastic cells now rendered harmless and re-embedded intact ground substance, subject again to normal tissue restraints, and persisting in the body in heterotopic situations as "paleoplastic" collections of essentially normal cells. A suggestion of the possibilities of the use of ascorbic acid in the control of cancer has been provided by the report by Schlegel and his collaborators (31) of its effectiveness against cancer of the bladder. It is our hope that a thorough trial will be given to this valuable substance, ascorbic acid, which may turn out to be the most valuable of all substances in the armamentarium of orthomolecular medicine.